**188-Pos Board B67**
**Unfolding, refolding and proteolysis of the von Willebrand Factor A2 domain under tensile force**
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von Willebrand Factor (vWF) is a plasma protein essential to the early stages of blood coagulation. Shear induced proteolysis at the A2 domain of vWF is an important mechanism to convert the highly thrombogenic, ultra large vWF multimers to smaller multimeric forms and, consequently, to prevent overgrown thrombus. It has been hypothesized that the A2 domain undergoes conformational changes in response to tensile force and exposes its Tyr842-Met843 scissile bond for cleavage by ADAMTS13, a metalloprotease found in the circulating blood. In this work, the unfolding and folding kinetics of the A2 domain is studied using optical tweezers under pulling forces that mimicked the tensile forces exposed to vWF multimers in the vasculature. We demonstrate that A2 domain is unstable upon pulling and unfolds at between 7 to 14 pN at loading rates ranging from 0.3 to 300 pN/s. Once unfolded, stress-free refolding of A2 domain takes 1.9 seconds, increasing dramatically with tensile force. Unfolded A2 domain was cleaved by physiological concentration of ADAMTS13 with a catalytic rate constant of 0.14/s. The results suggest that the A2 domain is unraveled at physiological tensile forces in vivo and its slow refolding process ensures the enzymatic reaction of ADAMTS13. Hence, the A2 domain acts as a force sensor that triggers ADAMTS13 cleavage at the pico-newton force range.

**189-Pos Board B68**
**The Temperature Dependency of Disulfide Bond Reduction Events Measured by Single-molecule Force Clamp Spectroscopy**
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An emerging application of single-molecule force clamp spectroscopy is the ability to explore the chemical kinetics of disulfide bond cleavage under different stretching forces at the single-bond level. Our previous work demonstrated that the rate of thiol/disulfide exchange reaction in proteins is force-dependent, and well described by an Arrhenius term of the form: $r = A(exp((F\Delta x_r\text{-}E_a)/k_BT)[nucleophile])$. From the force dependency of the reduction rate we could measure the bond elongation, $\Delta x_r$ (0.2-0.6 Å), that occurs at the transition state of the SN2 reaction cleaved by different chemical reagents and enzymes, never before observed by any other technique. However, our estimates of $E_a$ (53-60 kJ/mol) were uncertain and dependent on our choice of value for $A$ ($10^{12}$ $M^{-1}s^{-1}$). Here, we show that by carrying out the force-dependent disulfide bond reduction experiments at a series of well-controlled temperatures, $A$ can be measured independently. We demonstrate that the reaction rate of the disulfide bond cleavage by nucleophilic attack of tris(2-carboxyethyl)phosphine (*TCEP*) increases monotonically with temperature and, $A$ is measured to be at the order of $10^7$ $M^{-1}s^{-1}$, which is far lower than that predicted by the transition-state theory, in which $A$ is given by $k_BT/h$ and around $10^{12}$ $M^{-1}s^{-1}$ at room temperature. Factoring in the much lower value of $A$, $E_a$ is calculated to be 35 kJ/mol, which is much lower than 58 kJ/mol that we had reported previously. For thioredoxin (*Trx*)-catalyzed disulfide bond cleavage, obtaining the $A$, $E_a$ and $\Delta x_r$ values can help elucidating the reaction mechanisms and the role of temperature in the regulation of *Trx* activity, which is of special interest when comparing enzymes from different species. These measurements demonstrate the power of single-molecule force spectroscopy approach in providing unprecedented access to protein based chemical reactions.

**190-Pos Board B69**
**Direct Identification of Two Distinct Transition State Structures in Reduction of a Disulfide Bond Revealed by Single Bond Force-clamp Spectroscopy**
**Sergi Garcia-Manyes**, Jian Liang, Julio M. Fernandez.
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Disulfide bonds are common to many extracellular proteins, where they serve to stabilize the native conformation. Indeed, the thiol/disulfide exchange mechanism is involved in important and complex biological processes. Much experimental and theoretical work seems to support the idea that the reaction proceeds through an uncomplicated $S_N2$. While in gas phase a double minimum potential model describes the potential energy surface governing the chemical reaction, in solution phase only unimodal, concerted profiles without intermediates have been identified. However, the detailed shapes of the energy surfaces of these reactions are largely unknown, because the collisions with solvent control the trajectories of the molecules. The combination of molecular engineering techniques with single molecule force-clamp spectroscopy has made it possible to monitor the reduction of single disulfide bonds, allowing us to experimentally measure the bond elongation at the reaction transition state with sub-Ångström resolution. Such an experimental approach provides an unprecedented experimental platform to directly probe the energy landscape of a simple chemical reaction in solution at the single bond level. By greatly expanding the range of pulling forces up to 1.5 nN, where covalent bonds are not yet broken, here we demonstrate that the disulfide bond cleavage by hydroxyl occurs through a double-barrier energy landscape. Whereas at low pulling forces (100-500pN) the reaction rate is limited by a first energy barrier exhibiting a distance to the transition state $\Delta x$ ~0.5 Å, at higher forces (500-1500 pN) a second energy barrier exhibiting a shorter transition state of $\Delta x$ ~0.1 Å becomes dominant. Our experimental approach allows us to probe regions of the energy landscape that were previously experimentally inaccessible, revealing signatures of unanticipated complexity.

**191-Pos Board B70**
**Nebulin Elasticity Pre-loads Thin Filaments of Skeletal Muscle: Unfolding of Transient a-helices**
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Nebulin, a giant modular protein (600 - 900 kDa), acts as a thin filament ruler and regulator of contraction. The bulk of the protein consists of ~200 tandem repeats of ~35 residue actin-binding modules arranged as two single-repeat regions and 22 sets of seven-module super-repeats. The nanomechanics of nebulin were investigated with atomic force microscopy by tethering and stretching full length molecules between a pair of site-specific antibodies to either N or C terminus of nebulin, with one attached covalently to a functionalized self-assembled monolayer and the other to a functionalized cantilever. Upon stretch, single nebulin molecules extend to well over 1 μm and yield force curves with variable numbers and heights of peaks. An underlying periodicity at ~22 and ~15 nm was observed by periodogram analysis. Major force peaks appear to result from the re-orientation and unfolding of short α-helical bundles of nebulin modules, as suggested by circular dichroism, steered molecular dynamics simulations of computed structures as well as experimental force-extension curves of nebulin single and super repeats.
The nebulin molecule exerts a compressive force at the ends and alongside the 1 μm long thin filaments. In the sarcomere, nebulin's compressive stress may stiffen actin filaments and assist the re-annealing of severed actin filaments under undue stress. In the skeletal muscle sarcomere, the elastic nebulin associates helically around actin filaments, thus behaving as a length regulating ruler only under stress and upon binding to the actin filaments. It may also act as a reversible and elastic tether between the myosin heads and actin during contraction. We propose the elasticity of nebulin as an integral component of its ruler function and acto-myosin regulation in preloaded thin filaments.

**192-Pos Board B71**
**Effects of Cantilever Stiffness on Unfolding Force in AFM Protein Unfolding**
**William T. King**, Guoliang Yang.
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Direct mechanical unbinding of ligand-receptor pairs and the similar process of mechanically unfolding single protein molecules are common methods for exploring the energy landscape of unbinding/unfolding interactions. In these experiments, mechanical force applied through a cantilever and linking molecule causes the test system to transition between a bound/folded state and unbound/unfolded state. The effective linker stiffness k depends on both the chemical linker and the cantilever and is of central importance in calculating the loading rate $df/dt = kv$. This effect is well appreciated in standard analysis of unbinding/unfolding results. However, the effect of effective linker stiffness on the underlying energy landscape itself is often overlooked. Recent experiments on biotin-streptavidin by Walton et al. have shown surprisingly strong effects on average unbinding force under standard conditions, suggesting that future work should take the stiffness-modified landscape into account. We carry out force spectroscopy measurements on I27 octomers using cantilevers with a range of spring constants and discuss our findings and their effect on the analysis of mechanical protein unfolding experiments.

**193-Pos Board B72**
**E-selectin/sLea Form Catch-Slip Bonds Without Force-History Dependence**
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It has been established that P- and L-selectins form catch-slip bonds with their ligands, with initially increasing and subsequent decreasing bond lifetimes with

increasing load. Little, however, is known about the mechanics of E-selectin bonds with its ligands. We tested the load-dependence of bond rupture for E-selectin, and its dependence on the history of load application, by using the distribution of load between multiple receptor-ligand bonds to create a complex loading history. Briefly, an E-selectin-coated bead was held in a laser trap and touched to the vertical surface of a bead coated with sialyl lewis A ($sLe^a$), allowing one or more bonds to form with the ligand-coated surface. The laser trap was deflected away from the trapped bead, applying a nearly instantaneous load. When multiple bonds were present, we were able to discern the rupture of each bond as a step displacement of the trapped bead away from the stationary bead. In this way we were able to monitor both the number of bonds and the lifetime of each bond. We assumed that the load was evenly distributed between bonds and were thus able to monitor bond lifetimes across complex loading histories as the bonds ruptured asynchronously. Our data suggests that E-selectin/$sLe^a$ bonds behave as catch-slip bonds with critical forces of approximately 35 pN. Further, the lifetime of single bonds is similar to the lifetime of bonds that have previously shared load with others. This implies that E-selectin/$sLe^a$ bonds do not display strong force history-dependence. Rather, the bond lifetime is determined solely by the instantaneous load on the bond.

**194-Pos Board B73**

**How Protein Materials Balance Strength, Robustness And Adaptability**

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Proteins constitute crucial building blocks of life, forming biological materials such as hair, bone, spider silk or cells, which play an important role in providing key mechanical functions to biological systems. We present molecular dynamics simulations combined with theoretical modeling, used here to develop predictive multi-scale models of the deformation and fracture behavior of protein materials, capturing atomistic, molecular, meso- and microscopic scales. Through explicitly considering the hierarchical architecture of protein constituents, including the details of their chemical bonding, our models are capable of predicting their mechanical behavior across multiple length- and time-scales, thereby providing a rigorous structure-property relationship. We exemplify the approach in the analysis of two model protein materials, spider silk and intermediate filaments, representing beta-sheet and alpha-helix rich protein structures, respectively. Spider silk is a protein material that can reach the strength of steel, despite the predominant weak hydrogen bonding. Intermediate filaments are an important class of structural proteins responsible for the mechanical integrity of eukaryotic cells, which, if flawed, can cause serious diseases such as the rapid aging disease progeria or muscle dystrophy. For both examples, we present a multi-scale analysis that enables us to understand the structural basis of how these materials balance strength, robustness and adaptability through formation of hierarchical molecular and supermolecular features, and how structural flaws associated with genetic diseases contribute to the failure of these materials to provide biological function. We explain why the utilization of hierarchical features in protein materials is vital to their ability to combine seemingly incompatible properties such as strength and robustness, and adaptability. We discuss the implications of our work for the science of multi-scale interactions in biophysics, and how this knowledge can be utilized to develop de novo bioinspired nanomaterials via a bottom-up design.

**195-Pos Board B74**

**Single-Molecule Mechanics of the Muscle Protein Myomesin**

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Muscle contraction is mediated by molecular motors that interact between the thin and the thick filaments of muscle cells (actin and myosin, respectively). This leads to misalignment of the filaments within the sarcomeres, the otherwise highly ordered contractile subunits of muscle fibers. In order to maintain or restore the alignment, filament-crosslinking molecules must feature suitable elastic properties to tolerate and counteract tension forces. The M-band protein myomesin in the center of the sarcomere is such a crosslinker; it dimerizes and has binding sites for myosin and titin, the giant half-sarcomere spanning protein. Recent studies revealed that myomesin exhibits a so far unknown folding motif of immunoglobulin (Ig) domains that are connected by $\alpha$-helices (Pinotsis et al. 2008. EMBO J, 27(1):253-64); these presumably contribute to myomesin's physiological function.

Here, we report AFM force spectroscopy studies of myomesin which support the assumption that the Ig domain-connecting $\alpha$-helices augment the protein's overall tertiary structure elasticity with a previously unobserved secondary structure elasticity due to $\alpha$-helix unfolding and refolding at forces around 25pN. This particular behavior would allow myomesin to stay quite rigid up to an external tension force of about 25pN, while at higher forces the unfolding of the (fast refolding) $\alpha$-helices elongates the protein and thus protects the (slowly refolding) Ig domains from denaturation.

**196-Pos Board B75**

**Bending rigidity of type I collagen homotrimer fibrils**

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Normal type I collagen is an $\alpha1(I)_2\alpha2(I)$ heterotrimeric triple helix, but $\alpha1(I)_3$ homotrimers are also found in fetal tissues and various pathological conditions, e.g., causing bone fragility and reducing tendon tensile strength. It remains unclear whether $\alpha1(I)_3$ homotrimers alter mechanical properties of individual fibrils or affect tissues by altering their organization at a higher level. To address this question, we investigated how homotrimers affect fibril bending rigidity. Homotrimer fibrils have been shown to be more loosely packed so that we expected them to be more susceptible to bending. However, confocal imaging of *in vitro* fibrillogenesis revealed straight, spear-like homotrimer fibrils and curved heterotrimer fibrils. Surprisingly, homotrimer fibrils were more rigid despite being thinner and more hydrated. To quantify fibril rigidity, we analyzed their shape by Fourier decomposition, determined the correlation function for the direction along each fibril, and calculated the distribution of local fibril curvature. The fibril persistence length of homotrimers was 3 ~ 10 times longer than for homotrimers. These persistence length values indicated much higher bending rigidity of homotrimer helices. We conjectured that the increased rigidity might be related to stabilization of the region surrounding the mammalian collagenase cleavage site. In heterotrimers, this region is known to be the most flexible along the helix. We corroborated this hypothesis by probing the susceptibility of the collagenase cleavage site to MMP-1. Dissection of the observed effects revealed an increased stability of the homotrimer helix at this site. We argue that the loss of the $\alpha2(I)$ chain reduces type I collagen flexibility within the region most vulnerable to bending, thereby increasing the overall bending rigidity of the helix and fibrils. Higher fibril rigidity may alter tissue mechanics not only directly but also by changing the tissue scaffold architecture.

**197-Pos Board B76**

**AFM Manipulation Of Small Fibrin Networks**

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The mechanical properties of fibrin networks, the primary structural component of blood clots, are of great interest both from a biophysical and biomedical perspective. Macroscopic rheological studies have shown that, like other biopolymer gels, fibrin exhibits non-linear elasticity known as strain stiffening. The microscopic origins of this behavior are not well understood, however. We studied fibrin network mechanical properties using a combination fluorescence/atomic force microscope (AFM) system to quantitatively manipulate and visualize small (10-30 segments) two dimensional fibrin networks suspended over micropatterned channels. This setup enabled evaluation of the strain and orientation of each fiber in the network during AFM stretching manipulations as well as acquisition of force data. In other AFM manipulation work, we have shown that, like the macroscopic gels which they comprise, individual fibrin fibers exhibit significant strain stiffening. Our results show strain stiffening of individual fibers plays a significant role in the response of the overall network. In particular, strain stiffening affects the distribution of strain, reducing strain concentrations and spreading it more equitably throughout the network. In physiological contexts, this may act as a mechanism for strengthening the network and reducing the chance of mechanical failure (embolism). Our experimental data was compared to model networks of both linear and strain stiffening fiber segments.

**198-Pos Board B77**

**Probing Structure and Mechanics of Yeast Prion Proteins with Optical Tweezers**

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The relevance of amyloid fibers to a variety of severe human disorders and interest in using amyloid fibers as nanowire materials demands a better understanding of their molecular structures and mechanical properties. In Saccharomyces cerevisiae, protein Sup35, or its N-terminal fragment (NM) can assemble into a range of amyloid fibers with different underlying protein conformations. The structural diversity of Sup35 amyloids gives rise to a range of yeast prion phenotypes, referred to as weak [PSI+] or strong [PSI+]. This research uses combined optical trapping and fluorescence imaging to explore the mechanics, structure, and structural diversity of NM amyloid fibers. Fibers were reconstituted from purified fluorescently-labeled NM protein at 4°C and 37°C, which populate different protein conformations. These fibers induce strong